A Comparison of a Laparoscopic Kidney Transplantation Technique and an Open Approach in a Pig Model

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Abstract

Introduction: The expansion of laparoscopic surgery has emerged in the field of kidney transplantation as it confers multiple benefits. The aim of this study was to compare the outcomes of kidney transplantation by laparoscopic surgery versus open surgery in a pig model.

Materials and Methods: Sixteen large white pigs were included in this study. Ten pigs underwent laparoscopic kidney transplantation (Lap Group) and 6 pigs had open kidney transplantation (Open Group). Cooling of the kidney graft was introduced during vessel anastomosis. The time for vessel anastomosis was recorded. The kidney graft temperature and body temperature were monitored. Blood samples and biopsy of kidney graft were taken for analysis of kidney function.

Results: All surgeries were performed successfully. The time for renal artery anastomosis was longer in the Lap Group while the time for renal vein and ureter anastomosis was similar in both groups. The increase in kidney graft temperature was 3.6°C less in the Open Group than in the Lap Group. The creatinine level was similar at all time points and there was no difference in histopathology between two groups.

Conclusion: The kidney transplantation by laparoscopic surgery is safe and feasible. The kidney graft function was comparable in two groups. The laparoscopic technique has revealed a possible consideration for application to human orthotopic kidney transplantation in the future.

Keywords: Laparoscopy; Kidney graft; Kidney transplantation

Introduction

Laparoscopic surgery has been increasingly employed in clinical practice as it is minimally invasive with multiple benefits. More and more complex surgeries are now performed utilizing laparoscopic techniques [1-3]. Kidney transplantation by a laparoscopic technique has been explored over the last decade. Arguably, laparoscopic donor nephrectomy has set a benchmark and become the standard of care [4-6]. Previous studies have demonstrated that laparoscopic kidney transplantation is feasible and safe with benefits including improved cosmesis, less post-operative pain and shorter recovery periods [1,2,7,8]. However, a significant challenge of this technique is the time to perform the vascular anastomoses, which are longer than during conventional open surgery. This difference may predispose the kidney graft to ischemic reperfusion injury. The aim of this study was to compare laparoscopic and conventional open surgical techniques for kidney transplantation in a pig model.

Materials and Methods

The study was approved by the Animal Ethics Committee of The University of Western Australia, in accordance with the Code of Practice for the Care and Use of Animals for Scientific Purposes [9]. Sixteen female pigs (Sus scrofa, Large White Cross) were allocated to either of two groups: laparoscopic kidney transplant group (Lap Group, n=10) and open kidney transplant group (Open Group, n=6). The pigs were transported two weeks prior to surgery for acclimatization in the large animal facility at The University of Western Australia. The pigs were housed in raised communal...
pens (4 × 5 meters) with free access to tap water. A maintenance diet (Pig Grower, West Feeds Pty. Ltd., Australia) with fresh pumpkin and apples was provided. The room temperature was maintained at 22 ± 2°C and a 12/12 hour light/dark cycle. Environmental enrichment was provided with music, various toys and regular positive interaction with animal care personnel. Food was withheld for 12 h prior to surgery but free access to water was allowed. In addition, the pigs were examined by a veterinarian and were considered suitable for the study before the surgery.

**Anesthesia and Analgesia**

Pigs were anesthetized as previously described [10-12]. Briefly, anesthesia was induced with a combination of zolazepam and tiletamine (2 mg/kg of each drug in Zoletil 100, Virbac Australia Pty. Ltd., Australia) and xylazine (2 mg/kg, Ilum Xylazil 100 mg/mL, Troy Laboratories Australia Pty. Ltd., Australia) by Intramuscular (IM) injection. Subsequently, an auricular vein was cannulated to allow injection of propofol (1-2 mg/kg, Propofol Lipuro 1%, Braun, Germany). Endotracheal intubation was performed with a 7 mm internal diameter cuffed endotracheal tube (Portex, Smiths Medical, Minnesota, USA). General anesthesia was maintained with isoflurane (Attane Isoflurane, 1 mL/mL, Bayer, Australia) and glycopyrrolate (0.01 mg/kg IV Robinul, 0.2 mg/mL, Aspen Pharma Pty. Ltd., Australia) and xylazine (2 mg/kg, Ilium Xylazil 100 mg/mL, AstraZeneca Pty. Ltd., Australia) with animal care personnel. Food was withheld for 12 h prior to surgery but free access to water was allowed. In addition, the pigs were examined by a veterinarian and were considered suitable for the study before the surgery.

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Heparin (1500 IU) and mannitol (20 g) were administered intravenously (IV) 30 min prior to the occlusion of the renal artery by placement of the vascular clamp. In addition, frusemide (40 mg IV) was administered following kidney reperfusion. To facilitate surgical exposure pancuronium (0.1 mg/kg IV Pancuronium Injection BP 2 mg/mL, Astra Zeneca, Australia) was administered as required and neuromuscular blockade was monitored with a train-of-four pattern of stimulation (Innervator 252, Fisher and Paykel Healthcare, Auckland, New Zealand). Neostigmine (0.04 mg/kg IV Neostigmine injection BP, 0.5 mg/mL, AstraZeneca Pty. Ltd., Australia) and glycopyrrolate (0.01 mg/kg IV Robinul, 0.2 mg/mL, Aspen Pharma Pty. Ltd., Australia) were used to reverse neuromuscular blockade at the end of surgery. Analgesia was maintained by infusion of morphine (0.1 to 0.2 mg/kg/h) during surgery). Bupivacaine (0.5%, 20 mL) was infiltrated into the surgical wounds and laparoscopic port sites at the end of surgery. Post-operative analgesia was provided using tramadol (1 to 2 mg/kg IM) at the end of surgery and then every 12 h for 3 days; and morphine (0.1 to 0.2 mg/kg IM) every 6 h as deemed necessary in the post-operative period.

**Surgical Procedures**

The surgery consisted of two parts: laparoscopic left donor nephrectomy and subsequent orthotopic auto kidney transplantation by laparoscopic surgery (Lap Group) or open surgery (Open Group).

**Laparoscopic Left Donor Nephrectomy**

The pig was positioned in right lateral recumbency. The technique for laparoscopic left live donor nephrectomy was performed as previously described by our group [10,13]. Briefly, a camera port was first inserted via a small incision at the left para-midline, cranial to the umbilicus. Four other 12 mm ports were inserted under vision at the sites to form a “V” configuration (Figure 1a). The left kidney was identified and the peritoneum was incised at the renal hilum. The tail of the pancreas was mobilized superior medially. The renal vein was dissected followed by dissection of the renal artery and removal of the adjacent lymph nodes. Then the ureter was divided at the level of lower pole of the kidney and the kidney was mobilized from its attachment. At this stage, a small midline incision (8 cm) was made for delivery of the kidney graft in the Lap Group (Figure 1b); while a relatively longer incision (15 cm) was made at flank area for delivery of the kidney graft in the Open Group (Figure 2a, 2b). The laparoscopic vascular clamp was placed over the renal artery...
and renal vein respectively and the vessels were divided (Figure 3). The kidney graft was extracted and perfused with cold University of Wisconsin solution (4°C, 1L + 10,000 IU of heparin) on the back table and placed in an ice slush filled basin. The kidney graft was prepared and a marking suture was placed at the superior and inferior corner of the renal vein. The kidney was wrapped in a cold tailored surgical pack for transplantation.

**Ortho Topic Kidney Transplantation by Laparoscopic Surgery (Lap Group; n=10)**

Our technique for laparoscopic kidney transplantation has been described [10,12]. In this study, some modifications were made to improve surgical efficiency. Briefly, following preparation of the kidney graft on the back table, the kidney was delivered to the orthotopic position via the small midline incision (Figure 1b). The pneumoperitoneum was re-established. A different needle holder was used in this cohort of pigs than previously described by our group [10] (Figure 4). The anastomosis of the renal artery was modified using interrupted 6/0 Prolene sutures (Figure 5), whereas the renal vein anastomosis was performed by using two separate running 6/0 Prolene sutures, one for the posterior and one for the anterior component. The sutures were tied with a growth factor solution to prevent Stenosis (Figure 5). An additional refinement to the previous technique included application of a pump system for continuous irrigation of cold normal saline (4°C) to the kidney graft at the rate of 15 mL/min. Hemostasis was checked and the kidney was reperfused. The ureter was then anastomosed in an end-to-end fashion with 5/0 PDS interrupted sutures without placement of a ureteric stent. Lastly, the kidney graft was fixed in situ with 4/0 PDS suture at the upper pole, interpolar region and lower pole. Hemostasis was confirmed and the wounds were closed in layers. The pig was then repositioned in left lateral recumbency for ligation of the right ureter. The camera port was inserted by re-opening one of the 12 mm ports. A 5 mm and 12 mm port were inserted under vision. The right ureter was identified and ligated with Endo Clips (Covidien, USA) to ensure the ureter was completely occluded. The port sites were closed in layers.

**Ortho Topic Kidney Transplantation by Open Surgery (Open Group; n=6)**

In open surgery, the kidney graft was placed at the orthotopic location via the open flank incision (Figure 2a, 2b). The renal artery and renal vein were anastomosed in the same fashion as described in the Lap Group, but the approach was by conventional open surgery via a flank incision. Hemostasis was confirmed and the kidney was then reperfused. The ureter was anastomosed using 4/0 PDS suture by interrupted stitch. The kidney graft was fixed with 3 sutures at the upper pole, interpolar region and lower pole. The wounds were closed in layers. Ligation of the right ureter was also performed by the same technique as described in the Lap Group or via the same open incision.

In both groups, the time taken for anastomosis of the renal artery, vein, and the ureter was recorded. The temperature of the kidney graft was monitored by insertion of a thermometer probe into the kidney parenchyma when the kidney was in the ice slush, after completion of the renal artery and renal vein anastomoses respectively. Kidney biopsies were collected prior to donor nephrectomy, during the cold preservation, immediately post implantation and at the completion of the study 4 weeks post transplantation. Warm Ischemia Time (WIT) and Cold Ischemia Time (CIT) were recorded.

**Post-Operative Care**

Doppler Ultrasound (US) of the kidney graft was performed at the end of surgery to confirm vascularity of the kidney graft immediately after surgery. The pigs were recovered after surgery and observed closely for the first six post-operative hours. They were then observed twice daily for 14 days and once daily for another 14 days. Observations of respiratory rate and effort, gait, secretions from the eyes and nose, demeanour, eating and drinking, defecation and urination and appearance of the surgical wounds were performed and recorded. Blood samples were collected from the jugular vein immediately prior to and post surgery, and then on days 1, 3, 7, 14, 21 and 28 for full blood count, electrolytes, urea and creatinine levels.

**Euthanasia**

Four weeks following surgery the pigs were anesthetised as described above. A laparotomy was performed to examine the kidneys before tissue harvesting. The pigs were then euthanized by intravenous injection of pentobarbitone (160 mg/kg).
Histopathology

The kidney biopsies from 6 pigs (3 in each group) were processed, fixed in formalin, embedded in paraffin wax, and sectioned at 2 micrometres. Sections were stained with Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) respectively. The various renal compartments, including the glomeruli, tubules, interstitium, peritubular capillaries, arterioles and arteries were examined. Immunohistochemistry stains for T lymphocytes (CD3) were also performed.

Statistical Analysis

Data were tested for normality with a Shapiro-Wilk normality test. Study groups were then compared with an unpaired t test for specific time points or parameters and one-way analysis of variance within groups over time (GraphPad Prism 7, GraphPad 2016). Data are expressed as mean (SEM), p<0.05 was considered significant.

Results

All pigs were hemodynamically stable during the surgery without obvious blood loss. The surgery was performed successfully in all pigs. Normal waveforms of kidney graft vascularity were observed by Doppler ultrasound immediately post surgery (Figure 6a, 6b). The recovery in fourteen pigs was uneventful, while 2 pigs required early euthanasia. In the Lap Group, one pig was euthanized due to acute respiratory distress syndrome two hours after recovery from anesthesia. In the Open Group, one pig developed intra-abdominal sepsis secondary to urine leakage.

The time for renal artery anastomosis and total anastomosis time was greater in the Lap Group. There was no difference in the time for anastomosis of the renal vein or ureter. There was no difference in warm or cold ischemic time between groups (Table 1).

There was no difference in the temperature of the kidney graft prior to commencement of transplantation between the groups (4.7 ± 0.9°C vs. 5 ± 1.2°C, respectively). The kidney graft temperature increased to 26.6 ± 0.9°C in the Lap Group, and 23.2 ± 1.2°C in the Open Group in the time taken to complete the renal artery anastomosis. The subsequent increase in kidney graft temperature was less during the period of renal vein anastomosis (Table 2). The kidney graft temperature was lower in the Open Group than the Lap Group (25.2 ± 1.6°C vs. 28.8 ± 0.8°C, p=0.045) prior to kidney reperfusion. The body temperature remained stable during the period of vessel anastomosis (Table 2), during which time the kidney graft was continually rinsed with cold normal saline (4°C) at a rate of 15 mL/min. The body temperatures of the pigs were not different between the groups during surgery (Table 2). The body temperature of all pigs was higher at the time of kidney graft reperfusion when compared to the start of surgery in both groups (p=0.016).

The Hemoglobin (Hb) level remained stable before and after surgery and there was no difference between the two groups during the study (Table 3). The serum Creatinine (Cr) was elevated after completion of surgery in both groups. The peak creatinine levels within each group were observed on day 1 (272.4 ± 37.6 μmol/L in the Lap Group (p<0.0001) and 353.3 ± 48.3 μmol/L in the Open Group (p<0.0001), but there was no difference between the two groups on this day (Table 3). Kidney graft function continuously recovered over the remaining 4 weeks of the study. The serum Cr was 165.2 ± 17.6 μmol/L and 132.6 ± 12.1 μmol/L at the completion of the study in the Lap and Open Groups respectively (p=0.225) (Table 3).

Histopathology

There were no significant differences in the histopathological appearance of biopsies between the two groups (Figure 7a, 7b). Two pigs from the Open Group showed very mild acute tubular necrosis post-implantation, which had resolved histopathologically at completion of the study.

Discussion

This study demonstrates that kidney graft function following laparoscopic surgery is comparable to that following open surgery in a pig model despite a longer renal artery anastomotic time in the Lap Group. The refinement to the laparoscopic technique with a continuous cold irrigation system impeded the rewarming effect on the kidney graft during vessel anastomosis. This ongoing cooling relieved the tissue pressure experienced by the surgeon during vessel anastomosis. Other methods have been reported in the literature for cooling the kidney graft during vessel anastomosis for laparoscopic or robotic kidney transplantation [14-17]. In this pig model, the caliber of the renal artery is generally small with the diameter about 2 mm to 3 mm. Therefore, an interrupted suture technique is preferred for the renal artery in end to end anastomosis and tied with a growth factor solution that allows the vein to distend at the anastomosis site and prevent renal vein Stenosis.

Laparoscopic surgery has been expanding in clinical practice and replacing conventional open surgery, conferring multiple benefits [18-21]. Laparoscopic live donor nephrectomy is a platform through which laparoscopic surgery has emerged in the field of organ transplantation [22,23]. In open surgery for kidney transplantation, a large (15 cm to 20 cm) lower abdominal incision is required. As a result, wound complications have been reported with abdominal wall relaxation in 24% of cases; surgical site infection in 19%;
Table 2: Kidney and body temperature at the time of designated surgical events. P<0.05. Data are Mean (SEM).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Lap Group n=10</th>
<th>Open Group n=6</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney temperature prior to transplantation</td>
<td>4.7 (0.9)</td>
<td>5 (1.2)</td>
<td>0.826</td>
</tr>
<tr>
<td>Kidney temperature at completion of renal artery anastomosis</td>
<td>26.6 (0.9)</td>
<td>23.2 (1.2)</td>
<td>0.050</td>
</tr>
<tr>
<td>Kidney temperature at completion of renal vein anastomosis</td>
<td>28.8 (0.8)</td>
<td>25.2 (1.6)</td>
<td>0.045</td>
</tr>
<tr>
<td>Body temperature at start of surgery</td>
<td>35.1 (0.4)</td>
<td>35.4 (0.5)</td>
<td>0.604</td>
</tr>
<tr>
<td>Body temperature at completion of renal artery anastomosis</td>
<td>36 (0.3)</td>
<td>36.3 (0.6)</td>
<td>0.565</td>
</tr>
<tr>
<td>Body temperature at completion of renal vein anastomosis</td>
<td>36.3 (0.3)</td>
<td>36.5 (0.5)</td>
<td>0.679</td>
</tr>
<tr>
<td>Body temperature at reperfusion</td>
<td>36.1 (0.2)</td>
<td>36.3 (0.6)</td>
<td>0.708</td>
</tr>
</tbody>
</table>

Table 3: Blood tests of Haemoglobin and creatinine before and after surgery, P<0.05. Data are Mean (SEM).

<table>
<thead>
<tr>
<th>Blood test result</th>
<th>Lap Group n=10</th>
<th>Open Group n=6</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-operative sample</td>
<td>101.6 (2)</td>
<td>105.2 (4.4)</td>
<td>0.418</td>
</tr>
<tr>
<td>Post-operative sample</td>
<td>95 (4)</td>
<td>109.3 (6.2)</td>
<td>0.063</td>
</tr>
<tr>
<td>Day 1 sample</td>
<td>119.5 (5.4)</td>
<td>115.6 (4.4)</td>
<td>0.580</td>
</tr>
<tr>
<td>Day 3 sample</td>
<td>112.7 (6.7)</td>
<td>107.1 (4.9)</td>
<td>0.505</td>
</tr>
<tr>
<td>Day 7 sample</td>
<td>107.8 (3.7)</td>
<td>110.8 (2.2)</td>
<td>0.579</td>
</tr>
<tr>
<td>Day 14 sample</td>
<td>107 (2.9)</td>
<td>114 (3.7)</td>
<td>0.167</td>
</tr>
<tr>
<td>Day 21 sample</td>
<td>107 (4.5)</td>
<td>110 (4.5)</td>
<td>0.386</td>
</tr>
<tr>
<td>Day 28 sample</td>
<td>89.8 (4.7)</td>
<td>96 (3.4)</td>
<td>0.266</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-operative sample</td>
<td>94.4 (4.2)</td>
<td>89.3 (5.4)</td>
<td>0.470</td>
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<tr>
<td>Post-operative sample</td>
<td>113.2 (13.8)</td>
<td>125 (10.9)</td>
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<tr>
<td>Day 1 sample</td>
<td>272.4 (37.6)</td>
<td>353.3 (48.3)</td>
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<td>Day 3 sample</td>
<td>223.2 (28.1)</td>
<td>281.8 (17.7)</td>
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<td>Day 7 sample</td>
<td>152.6 (10.1)</td>
<td>151.2 (9.7)</td>
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<td>Day 14 sample</td>
<td>205.6 (36.1)</td>
<td>150.8 (10.5)</td>
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<td>Day 21 sample</td>
<td>202.3 (33.9)</td>
<td>140.6 (10.8)</td>
<td>0.241</td>
</tr>
<tr>
<td>Day 28 sample</td>
<td>165.2 (17.6)</td>
<td>132.6 (12.1)</td>
<td>0.225</td>
</tr>
</tbody>
</table>

incisional hernia in 16% and wound dehiscence in 4% [24,25]. The application of laparoscopic surgery for kidney transplantation would substantially reduce wound complications and promote recovery as only a small Pfannenstiel incision (7 cm) is needed for delivery of the kidney graft to the iliac fossa [2]. However, the implementation of laparoscopic surgery to clinical human kidney transplantation has been slow since the first report in 2010 [7]. The lag for adoption of this technique may be attributed to concerns for warm ischemic injury to the kidney graft during prolonged vessel anastomosis, in particular during the learning period for the surgeon. As far as medical ethics are concerned, mandatory training and perfecting the skill would ultimately protect the patient’s interest and achieve the optimal outcomes [26,27]. Surgeons have to face the challenge of surgical technique innovation under the concept of evidence-based medical practice. Animal and cadaver training models have provided a way to achieve the goal of creating a learning opportunity and an evidence basis for translation of the technique to human patients [28,29].

In addition, orthotopic kidney transplantation has not been widely practiced due to the degree of difficulty of surgical access. It requires a large flank incision through thick muscular layers or a long ventral midline incision. Consequently orthotopic kidney transplantation has been only considered in circumstances when the pelvic condition is not suitable for kidney transplantation such as occlusive iliac artery disease or previous multiple pelvic surgeries [30-32]. However, it is desirable for young kidney recipients to have the kidney graft placed in the orthotopic region if they are active and play sport involving physical body contact. This study has highlighted that this technique should be given serious consideration in human orthotopic kidney transplantation, in which only a small incision is required in this study, the surgical personnel had an opportunity to continually refine the technique. In situ cooling by employing a continuous irrigation system was introduced to impede the rewarming of the kidney graft. It is also important to acknowledge that the body temperature of the pig was stable during in situ cooling period. This method accommodates the surgeon’s requirement during the learning period for less time pressure to perform the vessel anastomoses by laparoscopy. The method employed in this study is simpler with a fluid pump to control the flow of cool fluid with regular suction and to avoid decreasing body temperature.

It is recognized that robotic kidney transplantation has been increasingly applied to clinical practice as it promotes efficiency of vessel anastomoses [33-35]. However, with the development of better laparoscopic instruments and devices, improvement in proficiency for vessel anastomosis is expected. In this study, a finer needle holder was employed allowing fingertip gripping (B Braun) for performance of the anastomoses, which was felt to have improved efficiency compared with the previous study [13].

From this study, it was also felt that the proficiency in performing this complex kidney transplant surgery improved over time. Consistency of the team members helped improve the performance of this challenging procedure. For the surgical assistant, it was vital to be able to hold the camera at the appropriate distance to facilitate a clear view for the vessel anastomosis and to adjust the needle. Moreover, the assistant was required to be familiar with two actions-exposure of the renal hilum and suction of the excess lavage fluid quickly while the surgeon was preparing the needle extra-corporeally.

In conclusion, continuous refinement of this laparoscopic technique for kidney transplantation in a pig model will promote translation of this technique to clinical practice in the near future. Application of continuous cooling to the kidney graft allowed amelioration of warm ischemic injury in the context of prolonged vessel anastomosis. The kidney graft function was comparable to open kidney transplantation. This animal model can be used for training in laparoscopic kidney transplantation.

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